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Quantum efficiency of Photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence *

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The balance between light-dependent reactions and electron-consuming reactions in intact sunflower leaves was varied by changing the incident light-flux at constant intercellular CO_2 concentration. Measurements of fluorescence quenching were compared to measurements of the rate and apparent quantum yield of whole-chain electron transport at a number of steady-state conditions. The steady-state quantum yield declined with increasing light intensity, falling at the highest intensity to approx. 40% of the maximum value observed in low light. The coefficient for photochemical quenching, q_Q, was near 1 in low light and only declined to 0.7 at the highest light, indicating that there was very little feedback from accumulation of reduced electron carriers. On the other hand, there was a large increase in q_E, the coefficient for 'energy'-dependent quenching, as the quantum yield fell. We found that these changes in the steady-state quantum yield, Φ_s, could be related to the changes in fluorescence quenching by an empirical equation, $\Phi_s = q_Q(0.32 - 0.17\,q_E)$ which accounted for variation in Φ_s resulting from light saturation or changes in CO_2 concentration. We develop a hypothesis that Photosystem (PS) II centers may be converted to an altered state (possibly mediated by the chloroplast ΔpH) which has very little variable fluorescence and a lowered photochemical yield. We develop a kinetic explanation for the properties of the altered form of PS II, and we propose that this mechanism (indicated by q_E) functions together with the accumulation of reduced Q_A (indicated by q_Q) to regulate the rate of net photochemistry by PS II when — with increasing light or decreasing CO_2 — the potential rate of net photochemistry exceeds that for carbon metabolism. The latter mechanism apparently permits down-regulation of PS II to occur without strong accumulation of reduced Q_A, except during transients or under the most extreme conditions.

Introduction

Quanta absorbed by pigments associated with PS II may be used to do photochemical work, may be transferred to PS I, or may be dissipated as heat or fluorescence. Changes in environmental factors such as the light intensity, CO_2 concentration or temperature which control photosynthesis must affect the balance between these processes resulting in changes in both the steady-state quantum yield for net electron transport and

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4'-dichlorophenyl)-1,1-dimethylurea; Q_A, primary electron acceptor of PS II; PAR, photosynthetically active radiation (400–700 nm); PS, Photosystem; P-680, the reaction center chlorophyll of PS II.

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changes in the yield of chlorophyll fluorescence. Fluorescence, therefore, provides a useful, although generally qualitative indicator of the reactions of PS II. This paper reports on studies which show that measurements of fluorescence during steady-state photosynthesis can be used to obtain quantitative estimates of the apparent quantum yield for net electron transport. The studies were conducted with attached leaves enclosed in a cuvette fitted for measurement of net CO_2 exchange and chlorophyll fluorescence. The steady-state quantum yield varied as the intensity of illumination increased from 'rate-limiting' to 'rate-saturating' levels.

It is generally accepted that variations in the yield of chlorophyll fluorescence in vivo can occur as a result of changes in the redox level of the primary electron acceptor of PS II, (photochemical quenching) or by a nonphotochemical mechanism which seems to be related to acidification of the inner thylakoid space and related structural changes that occur upon 'energization' of the chloroplast membrane system [1], for a review see Ref. 2. Early work by Delosme et al. [3] and subsequent studies [4–6] showed that changes in the apparent yield for photochemistry were complementary to changes in fluorescence yield where only the photochemical mechanism of quenching varies (for example, during the induction of photosynthesis in the presence of DCMU [4]). However, it has been difficult to apply this relationship to normal steady-state photosynthesis since 'energy'-dependent quenching does not remain constant as environmental conditions are changed. When photosynthesis approaches 'light saturation,' the level of 'energy'-dependent quenching tends to increase and the level of photochemical quenching tends to decrease [7]. The resulting changes in fluorescence yield are in opposing directions, and have been difficult to resolve (cf. Ref. 8). An experimental procedure described recently by Schreiber [9], based on progress in analysis of steady-state fluorescence [30,31], permits resolution of quenching by each of these mechanisms under any steady-state condition. This opens new possibilities for quantitative interpretation of fluorescence.

The experimental approach used to examine these quenching mechanisms is discussed in detail elsewhere [10]. Briefly, chlorophyll fluorescence is excited with a low-intensity modulated light and is detected with a fluorometer which is selective for this modulated fluorescence. This permits monitoring of the yield of fluorescence independent of the intensity of background illumination. The quenching mechanisms considered here primarily affect the yield of variable fluorescence. For a given leaf, the maximum variable fluorescence is obtained upon application of a saturating pulse of light when the leaf has been pre-conditioned to darkness for several minutes, yielding a reference level, $(F_v)_m = F_m - F_0$, which is used to evaluate quenching of variable fluorescence under other conditions. As used here and defined in ref. 10, quenching is quantified by a coefficient ($0 < q < 1$), where the variable fluorescence at steady-state, $F_v = (1 - q) \cdot (F_v)_m$. The total quenching can be subdivided into component coefficients q_Q and q_E, indicating extent of quenching caused by photochemical and 'energy'-dependent mechanisms, respectively. These are defined such that $F_v = (F_v)_m \cdot (1 - q_E) \cdot (1 - q_Q)$.

The protocol for separating q_Q and q_E takes advantage of the fact that the redox level of the PS II acceptor (which regulates photochemical quenching) responds very rapidly to changes in the light regime, while changes in the ion and pH gradients (which govern 'energy'-dependent quenching) are much slower. The photochemical component of quenching is determined by referencing the steady-state F_v to a level $(F_v)_s$ obtained by rapid manipulations of the light regime to obtain the F_0 and F_m levels at the steady-state while not permitting the 'energy'-dependent mechanisms to relax. If 'energy'-dependent quenching is occurring under this condition, the intermediate reference level, $(F_v)_s$ will be lower than $(F_v)_m$.

We report here an analysis of steady-state photosynthesis which considers the influence of both quenching mechanisms. This analysis is based upon the premise that the photochemical yield should be proportional to changes in q_Q. We then examine the remaining variation for its relation to q_E. This analysis provides support for recent work indicating that the photochemical yield may be reduced by mechanism that result in changes in 'energy'-dependent quenching of fluorescence [11–13].

Materials and Methods

Attached leaves were mounted in a leaf chamber equipped to measure gas exchange as described in Ref. 14 and chlorophyll fluorescence as described in Ref. 7. White actinic light was provided for photosynthesis, and a weak, modulated, measuring light (approx. 1 $\mu E \cdot m^{-2} \cdot s^{-1}$) was provided by a fiberoptic, which also collected the fluorescence. This was analyzed by a pulse amplitude modulation fluorometer [9] (PAM-101, H. Walz, D-8521 Effeltrich, F.R.G.). The measuring system selectively monitors the fluorescence yield of the modulated light, and is not effected by normal levels (up to 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR) of white actinic illumination (L_1) or by high intensity (> 4000 $E \cdot m^{-2} \cdot s^{-1}$) pulsed illumination ($L_2$). L_1 was used to drive steady-state photosynthesis; L_2 was pulsed to obtain transient reduction of the photochemical traps at the steady state, and a far-red light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$, > 720 nm), L_3, could be applied in the absence of white light to oxidize the intersystem electron carriers.

The reference level, $(F_v)_m$, was obtained with the leaf after at least 5 min of dark pretreatment as the difference between the F_0 (measuring light plus a brief pulse of L_3) and the F_m (during a pulse of L_2). For each experimental condition, the level of fluorescence was recorded when the leaf had reached a steady-state at a constant intensity of L_1 (generally after 30–60 min). Then the maximum fluorescence (F_m) yield was recorded when a saturating pulse of L_2 was provided in addition to L_1. Finally, the minimum fluorescence yield (F_0) was recorded during short periods when L_1 was interrupted in the presence of L_3. The F_0 level was subtracted giving yields of variable fluorescence at the steady-state, F_v, and during the saturating pulse, $(F_v)_s$. The coefficients were obtained essentially as in ref. 10, except that the F_0 level used was the minimum obtained (in the presence of far red light to oxidize intersystem electron carriers) within approx. 2 s of interrupting light L_1 from the steady-state conditions. This level could be up to 15% lower than the F_0 level obtained with the leaf in a dark-adapted state. The coefficient for photochemical quenching was taken as, $q_Q = 1 - F_v/(F_v)_s$. The coefficient for non-photochemical quenching as $q_E = 1 - (F_v)_s/(F_v)_m$, and it is useful to note that the relative yield of variable fluorescence, $\Phi'_{F_v} = (F_v)_s/(F_v)_m = 1 - q_E$.

The gas-exchange experiments were generally conducted in 2% O_2 to minimize corrections for O_2-reducing reactions, and the rate of net electron transport (J) was calculated from the rate of gross CO_2 exchange (net uptake plus 'day' respiration), and estimates of the concentrations of CO_2 and O_2 in the chloroplasts according to Eqn. A8 of Ref. 15.

Results

Fig. 1 shows a plot of the rate of electron transport, J, of an intact leaf of a sunflower plant as a function of I, the incident quantum flux (PAR), at a constant intercellular CO_2 concentration (approx. 200 ppm). The leaf was permitted to come to steady-state at each value of I, then the levels of fluorescence and the rate of CO_2 assimilation were recorded. Net electron transport, J, was calculated as in Ref. 15. With increasing I, J becomes less sensitive to I and ultimately saturates. Other experiments (data not shown) indicated that this rate could be increased by increasing the CO_2 concentration. Thus, the capacity of carbon metabolism to provide electron acceptors presumably controls J at 'light-saturation' in these experiments.

The steady-state level of variable fluorescence was very low, as shown by the coefficient for total

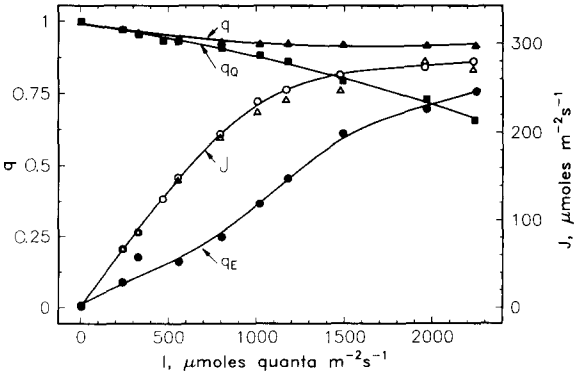

Fig. 1. Light-response of the photosynthetic electron transport, J, calculated from measurements of net CO_2 exchange (○) or from Eqn. 1 (△), and coefficients for fluorescence quenching, q_E (●) and q_Q (■), of an intact *H. annuus* leaf.

quenching, q (Fig. 1), which ranged from 1.0 to 0.9 over the range of light intensities studied. However, there were large changes in the proportion to which this quenching could be attributed to 'photochemical' or 'energy'-dependent mechanisms as indicated by q_Q and q_E, respectively (Fig. 1). In low light, quenching was almost entirely photochemical in nature. The coefficient, q_Q, decreased as the electron transport rate increased. This corresponds to an increase in the ratio $F_v/(F_v)_s$ at the steady-state, and presumably indicates an increase with increasing I, in the proportion of PS II traps having a reduced primary acceptor, Q_A [7]. Even at very high light intensity, the photochemical traps apparently remained mostly in the 'open' configuration.

'Energy'-dependent quenching was insignificant in low light, and it increased as the rate of electron transport increased. (This corresponds to a decrease in the maximum yield of variable fluorescence, Φ'_{F_v}, from that observed in the dark). The largest changes in q_E occurred over the range of I where 'light saturation' was reached, and this change was presumably related to a large ΔpH that is built up under such conditions [7]. We also noted a decrease in the F_0 level that appeared to be correlated with the increase in q_E quenching at high light. This effect has also been noted by Bilger and Schreiber [16], who define a coefficient q_0 for this. In our studies, $q_0 \approx 0.15$ when $q_E = 0.8$, but values of $q_0 > 0.25$ have been observed with other species (data not shown). We have taken these changes in F_0 into account in calculating the values of q_Q and q_E, and possible complications caused by changes in F_0 are considered in the Appendix.

In Fig. 2 we compare the levels of fluorescence quenching and the apparent steady-state quantum yield (based upon incident light) for net electron transport-defined here as $\Phi_s = J/I$, and calculated for each steady-state condition from the data shown in Fig. 1. As noted previously [7], the decrease in quantum yield correlates with a decline in photochemical quenching, q_Q. However, over the range studied, the change in q_Q was small compared to the change in Φ_s. On the other hand, there was a large increase in q_E. These obervations are similar to those of Krause and Laasch [12] and Weis et al. [13], who suggested that the photochemical activity of PS II in vivo may be regulated (in part) by non-photochemical quenching of excitation energy, indicated by an increase in q_E.

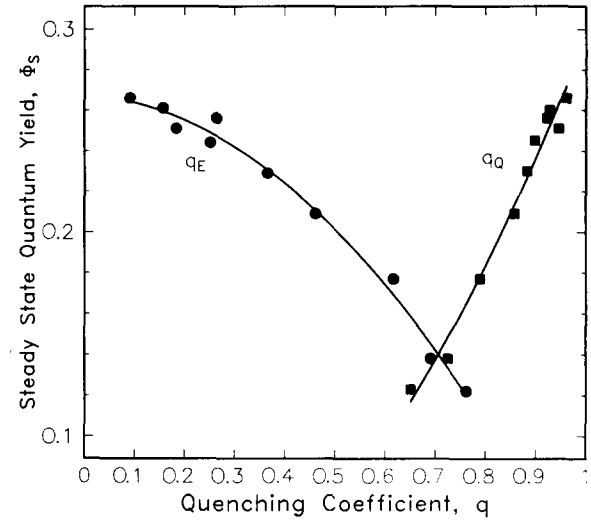

Fig. 2. The dependence of the apparent steady-state quantum yield (for incident light) of net electron transport (Φ_s) on the coefficients q_E and q_Q. Calculated as $\Phi_s = J/I$ from the experiment shown in Fig. 1.

To test this possibility, we 'corrected' the quantum yield at each steady state according to the level of q_Q yielding an estimate, Φ_P, of what the quantum yield would have been, had the traps been fully open (i.e., Φ_P should be independent of the accumulation of Q_A^-). This parameter varied between two extremes, (Φ_{P_0} and Φ_{P_c}, at $q_E = 0$ and 1, respectively; Fig. 3). Φ_{P_c} was lowered by more than 50% compared to Φ_{P_0}, and Φ_P was highly correlated with q_E. Similar results were observed in other experiments when the balance between carbon fixation and light-dependent reactions was altered by changing the CO_2 concentration at constant light (data not shown).

In order to compare this result to theoretical calculations, we have plotted these data and those from a similar experiment with *Phaseolus vulgaris* as the normalized yield for photochemistry Φ'_P against that for variable fluorescence from PS II, $\Phi'_{F_v} = 1 - q_E$ (Fig. 4). As shown, the species *Helianthus annuus* and *P. vulgaris* differed in the slope and intercept of the empirical relationship. The difference between species was consistently seen in other experiments. Fig. 4 also shows a schematic diagram of Butler and Kitajima's bipar-

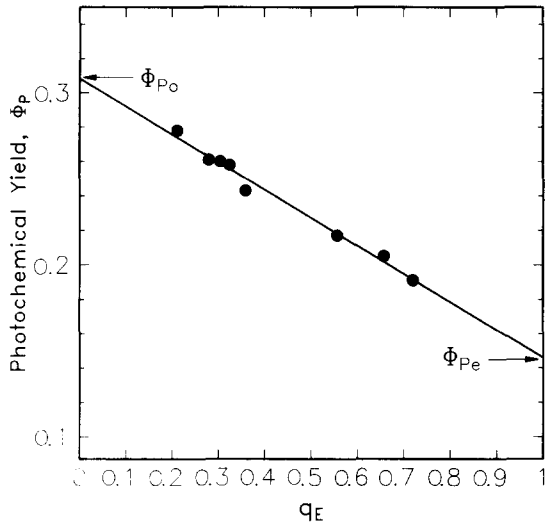

Fig. 3. The dependence of the quantum yield with 'open' PS II centers (Φ_P) on q_E. Calculated as $\Phi_P = \Phi_s/q_Q$ from the experiment shown in Fig. 1.

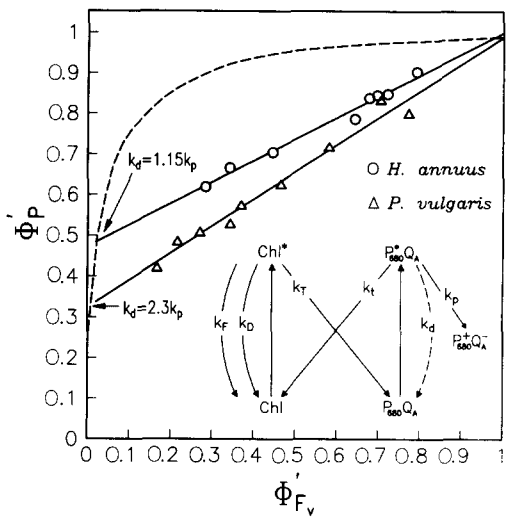

Fig. 4. The normalized quantum yield of 'open' PS II centers and the level of variable fluorescence (difference between the 'closed' and 'open' states) for a single PS II center calculated according to the bipartite model [17] (dashed line) with experimental data from Fig. 1 and from a similar experiment with *P. vulgaris*. The solid lines are linear regressions of the data. The inset shows a schematic diagram of the bipartite model. The kinetic constants are: k_F, radiative decay; k_D, radiationless decay in the antenna; k_T, energy transfer from antenna to reaction centers; k_t, backtransfer from reaction centers to antenna; k_p, photochemistry, and k_d, radiationless decay at the reaction center. Equations used for the calculations are given in the Appendix. Calculated values of Φ'_P were normalized on $\Phi_P = 0.86$ at $k_d = 0$, and measured values of Φ_P were normalized on $\Phi_P = 0.32$ at $q_E = 0$ (see Fig. 3).

tite model [17], relating fluorescence and photochemistry to the kinetics of processes occurring either in the antenna or at the reaction center of PS II. The results of calculations based on that scheme are displayed (dotted line, Fig. 4).

The result that Φ_s/q_Q is highly correlated with q_E indicates that the steady-state quantum yield can be related to the levels of photochemical and non-photochemical quenching by an equation of the form:

$$\Phi_s = q_Q(q_E\Phi_{P_e} + \Phi_{P_0}(1 - q_E)) \quad (1)$$

where Φ_{P_0} and Φ_{P_e} are obtained from the intercepts at $q_E = 0$ and 1 (Fig. 3), or by linear regression analysis of the form, $J/q_Q I = b - mq_E$, where $b = \Phi_{P_0}$ and $m = \Phi_{P_0} - \Phi_{P_e}$. The fit of this relationship to the experimental data is illustrated in Fig. 1, which compares the electron transport rate calculated from the fluorescence quenching measurements as $J = I \cdot \Phi_s$ using Eqn. 1 (Δ, Fig. 1) to values measured by gas exchange (O, Fig. 1). A more complete test is provided in Fig. 5, which summarizes data obtained in several experiments where light, temperature or CO_2 were manipulated to vary the balance between light-dependent and electron-consuming reactions with both *P. vulgaris* and *H. annuus*. The electron-transport rate estimated from measurements of fluorescence and incident quantum flux were very well correlated with the rate of electron transport required to support the measured flux of CO_2 uptake. It is particularly important that the correlation held when CO_2 was kept constant and light was varied, or when light was kept constant and CO_2 was varied — excluding the possibility of a secondary correlation with light intensity.

Discussion

The analysis of fluorescence quenching reported here leads us to conclude that mechanisms which regulate the level of non-photochemical quenching in vivo play a very important role in regulating the rate of PS II photochemistry at steady-state photosynthesis. This becomes particularly important when the incident light flux is more than sufficient to drive the maximum rate of

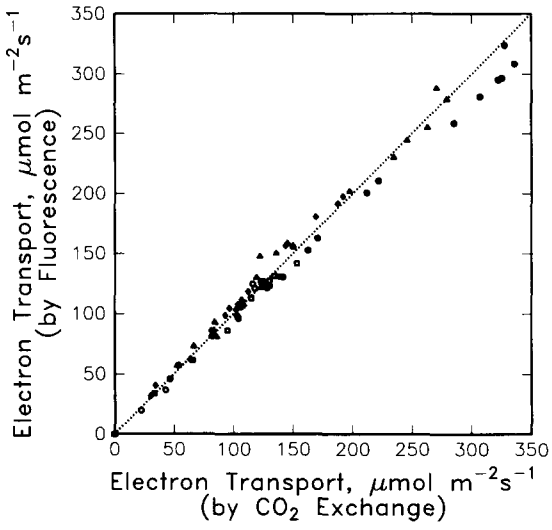

Fig. 5. A comparison of measurements of electron transport rate for intact leaves of *P. vulgaris* and *H. annuus* conducted concurrently by CO_2 exchange or by analysis of fluorescence quenching. Fluorescence measurements were related to electron transport rate by $J = I \cdot q_Q(\Phi_{P_e} \cdot q_E + \Phi_{P_0}(1 - q_E))$ where q_Q and q_E are the coefficients for photochemical and non-photochemical quenching evaluated at the steady-state and Φ_{P_0} and Φ_{P_e} are the quantum yields (on the basis of incident light, I) for the normal and the 'quenched' forms of the PS II center. $\Phi_{P_0} = 0.32$ for both species and $\Phi_{P_e} = 0.150$ and 0.100 for *H. annuus* and *P. vulgaris* respectively. These were determined by regression analysis. The different symbol types are each for a separate response curve such as shown in Fig. 1. These include light response curves at different temperatures, a CO_2 response curve and one light response curve done in normal oxygen.

CO_2 fixation. More than 20 years ago, Duysens and Sweers [18] speculated that variation in non-photochemical quenching observed in vivo was under the control of a 'dark reaction,' and that it "traps excitation energy of the unit which otherwise might cause photo-bleaching of the chlorophyll a_2 or other harmful effects". Our results provide direct support for this idea. It is particularly significant that this appears to prevent strong accumulation of Q_A^-, except under the most extreme conditions. This may implicate q_E quenching as mechanism that would protect against photoinhibition (cf. Refs. 11, 12 and 19). Furthermore, as will be discussed in a subsequent paper, this type of regulation may be very important in poising the redox level of the intersystem electron carriers which participate in cyclic electron flow around PS I.

The quantitative analysis of fluorescence quenching has led to the development of a very robust empirical expression which provides a useful estimate of the electron transport rate, J from measurements of the incident quantum flux, I, and the quenching coefficients, q_Q and q_E (Fig. 5). We note that this empirical relationship provides a means of conducting measurements of photosynthetic activity using optical methods which do not require the enclosure of the plant material to be studied, and that this could overcome a major limitation to studies of photosynthesis of plants in their natural environments. The rate of net CO_2 assimilation and perhaps even the stomatal conductance [20] are driven by electron transport.

The success of this empirical analysis also leads us to suggest that it may provide new insight into the processes which control PS II photochemistry in vivo. The first step in the analysis presented here assumes that $\Phi_s = q_Q \cdot \Phi_P$ under any condition, provided q_E remains constant. There is strong theoretical and experimental support for the assumption that photochemistry is proportional to q_Q (at least for conditions where $q_E = 0$, see Refs. 3–6). Using q_Q to calculate the effect of reduced Q_A on the quantum yield for net photochemistry, does not account for all variation in the steady-state quantum yield (Fig. 3), and the remaining variation was highly correlated to the coefficient, q_E. If the first step in our analysis is valid, then this shows that there is a linear decline in the apparent quantum yield of open PS II centers when 'energy'-dependent quenching of fluorescence, increases (Figs. 3 and 4).

There is no reason to expect a priori that the photochemical yield should vary linearly with changes in 'energy'-dependent quenching of F_v. The bipartite model of Butler and Kitajima [17] (see inset, Fig. 4) can be used to examine the possible kinetic basis for this result. This model allows non-photochemical, non-radiative de-excitation to occur either by reactions occurring in the antenna pigments characterized by a rate constant, k_D, or by reactions occurring at the reaction center characterized by k_d. These may be differentiated because quenching at k_D quenches both F_0 and F_v, while quenching at k_d mostly affects F_v [17]. 'Energy'-dependent quenching

seems to be of the k_d type, since variable fluorescence is more strongly affected than is F_0. Therefore, we calculated the expected changes in the relative yields of variable fluorescence, Φ'_{F_v} and photochemistry, Φ'_P assuming that k_d of a single, isolated center was changed while keeping all other rate constants fixed (at plausible values, see Appendix). Results of these calculations (normalized to give a maximum $\Phi'_{F_v} = 1$) are plotted (dotted line, Fig. 4) together with normalized experimental data. The strongly non-linear relation between fluorescence yield and photochemical yield predicted by this calculation contrasts with linear relationship observed with experimental data.

One way to account for our results within the bipartite model is to postulate that k_d does not change continuously for all PS II centers, rather there may be different forms of PS II with distinctly different values of k_d, and as a consequence different yields, Φ'_{F_v} and Φ'_P. We propose that the correlated changes in fluorescence yield and photochemical yield is the result of a regulatory mechanism which controls the interconversion between these forms. Other workers have recently suggested that PS II may be heterogeneous [6,21,22], but it is not clear how these observations may relate to the model proposed here. We note that the quenched form of PS II proposed here is essentially similar to the hypothetical Q′ proposed by Duysens and Sweers [18].

To be consistent with the experimental data the Φ'_{F_v} and Φ'_P of the quenched centers must fall along the lines drawn through the experimental data points (Fig. 4), and to be consistent with the bipartite model the yields must lie along the dashed line. These lines intersect indicating solutions consistent with both the bipartite model and our empirical observations.

According to this hypothesis, the PS II centers of a leaf could be in one of four possible states, depending on the redox level of the plastoquinone pool and depending on the mechanism which regulates the 'high-energy state', as shown:

$$\begin{array}{ccc} \text{PS II}_0 & \rightleftharpoons & \text{PS II}_0 \\ \updownarrow & & \updownarrow \\ \text{PS II}_e & \rightleftharpoons & \text{PS II}_e^- \end{array}$$

where PS II_0 and PS II_e are the 'open' configuration of the 'normal' and the 'quenched' forms of PS II, while PS II_0^- and PS II_e^- are the 'closed' configurations (with Q_A^-), respectively.

Changes in the extent of quenching of chlorophyll fluorescence by photochemical or 'energy'-dependent mechanisms observed in going from one steady-state condition to another, or during induction of photosynthesis (cf. ref. 10) may be interpreted as indicating changes in the proportion of PS II in these four forms. These changes should be controlled by the redox level of the plastoquinone pool and by the 'energization' of the chloroplast membrane system, and we suggest that these changes are the result of feedback loops which regulate the rate of whole-chain electron transport to match the rate at which electrons can be accepted by linked biochemical reactions.

We defined partial coefficients which indicate the relative proportion of each of the four proposed states. At any steady-state, there may be: a, PS II_0; b, PS II_0^-; c, PS II_e, and d, PS II_e^-, where $a + b + c + d = 1$. These coefficients could then be related to fluorescence and photochemical yields for isolated PS II centers in the different states, as calculated on the basis of equations developed by Butler and Kitajiama [17]. Results are shown in Table I and the calculations are explained in the Appendix. The quanta absorbed by each of the four states should be directly proportional to its concentration, but because of net energy transfer between centers, the proportion of quanta de-ex-

TABLE I

CALCULATED ACTUAL AND NORMALIZED YIELDS FOR FLUORESCENCE Φ_{F_x} AND PHOTOCHEMISTRY Φ_{P_x} (WHERE x MAY BE a, b, c OR d TO INDICATE THE CORRESPONDING STATE) FOR THE FOUR STATES OF PS II CENTERS PROPOSED IN THIS MODEL

Refer to the Appendix for a description of the calculations. Normalization was conducted to give a maximum $\Phi'_P = 1$ and a maximum variable fluorescence, $\Phi'_{F_b} - \Phi'_{F_a} = 1$.

	PS II_0 (a)	PS II_0^- (b)	PS II_e (c)	PS II_e^- (d)
Actual yield				
Φ_{F_x}	0.0255	0.167	0.0209	0.0226
Φ_{P_x}	0.893	0	0.338	0
Normalized yield				
Φ'_{F_x}	0.180	1.180	0.148	0.160
Φ_{P_x}	1.000	0	0.380	0

cited by a particular center (and consequently its fluorescence or photochemical activity) may be somewhat more or less than it absorbs. For simplicity, we will ignore here transfer of excitation energy, and we will attribute all changes in variable fluorescence to changes in the coefficient b, the proportion of PS II_0^-. But for certain applications (for example, correlation with optical measurements of Q_A or the ΔpH) it may be necessary to correct for energy exchange. Therefore, a more rigorous treatment of energy exchange in terms of the model proposed here is presented in the Appendix.

We can now consider the meaning of q_Q and q_E in terms of this model and relate these quenching coefficients to the partial coefficients defined above. The reference state where $\Phi'_{F_v} = 1$ is the maximum variable fluorescence observed in the dark-adapted state, $(F_v)_m$. We assume that the PS II_0 form is the only form present in the dark-adapted state. Thus, $F_m - F_0$ represents total conversion of all PS II centers from the PS II_0 to the PS II_0^- forms, and b (in L_2) = 1. With an illuminated leaf, $(F_v)_s$ is obtained in the analogous way to $(F_v)_m$, except that some of the centers may now be in the PS II_e forms, and the total yield of variable fluorescence will be correspondingly lower because $b < 1$. At steady-state, $(F_v)_s = a + b$, and $q_E = 1 - (F_v)_s/(F_v)_m = 1 - (a + b) = c + d$. Similarly, $q_Q = 1 - F_v/(F_v)_s = 1 - b/(a + b)$, or $q_Q = a/(a + b)$. If we assume that the centers are in equilibrium with the same plastoquinone pool (i.e., $a/(a + b) = c/(c + d) = q_Q$), we have sufficient information to calculate proportion of any center of interest. For example, $b = (a + b) \cdot b/(a + b) = (1 - q_E)(1 - q_Q)$, and $F_v = b(F_v)_m = (1 - q_E)(1 - q_Q)(F_v)_m$, as defined in Ref. 10.

The total yield for photochemistry of PS II can be calculated, knowing the respective quantum yields of the two 'open' forms (Table I), and a and c, where $a = (1 - q_E)q_Q = (a + b) \cdot a/(a + b)$, and $c = q_E \cdot q_Q = (c + d) \cdot c/(c + d)$. Therefore,

$$\Phi_s = q_Q(q_E\Phi_{P_e} + \Phi_{P_0}(1 - q_E))$$

This equation is identical to Eqn. 1.

Based on this approach, we could simulate transformation between the four states, fluorescence quenching and photochemistry in a hypothetical leaf. This is explained in the Appendix. We conclude that the apparent fluorescence quenching coefficients can provide an adequate measure of the distribution of energy among the different centers.

This hypothesis provides a logical explanation for the strong empirical correlation established between the steady-state quantum yield and the quenching coefficients determined at the steady-state. The present development does not provide a rigorous treatment of the fluorescence yield of each of the centers and it ignores the interactions between centers in a heterogeneous mixture. The errors caused by these simplifications (as shown in the Appendix) are not large.

For a wide range of conditions, it may be reasonable to assume that Q_A becomes almost completely reduced, when a light pulse is given. However, when a high electron flux is established, high light intensity and high turnover of PS II during the light pulse may be required to overcome the re-oxidation of Q_A. The efficiency of a light pulse to reduce Q_A could also be affected by a possible limitation of the activity of PS II by the donation of electrons from the water-splitting site. Another assumption is that the PS II_e centers are affected to a similar extent to the PS II_0 centers by accumulation of reduced plastoquinone, but the measured value of q_Q only reports on the PS II_0 centers, since only these emit variable fluorescence. These questions may require further examination (cf. Ref. 22).

It is interesting to note that the theoretical calculations (Table I) predict a slightly reduced F_0 when the centers are mostly in the PS II_e form. This may explain our observation that F_0 is also quenched when q_E is large. The extent of F_0 quenching depends upon the ratio of k_T/k_t (transfer to the reaction center from the antenna, and the backtransfer). Our calculations take into account recent evidence [25] that there may be significant backtransfer.

It is not yet clear, what mechanisms lead to non-photochemical quenching at the reaction centers of PS II. Recently, Oxborough and Horton [26] have suggested that the link between ΔpH and 'energy'-dependent quenching may not be

obligatory, and that redox reactions might also be involved. Our hypothesis does not depend on any specific mechanism of quenching. We also note that several quenching processes could contribute to the general phenomenon of 'high-energy' quenching. Other processes, such as quenching by compounds that react with the antenna pigments [17] and effects of photoinhibition [19], may have influences not considered here. It also requires further examination to understand the relationship between the present model and state transitions caused by phosphorylation of the light-harvesting complex and regulating the balance of excitation energy between PS I and PS II [23,24]. Recent studies suggest that upon protein phosphorylation a high-fluorescent 'PS II_α' form is converted into a low-efficient and low-fluorescent 'PS II_β' form [32,33]. Interestingly, PS II_β resembles, in some respect, the PS II_e form proposed in this study. But apparently, only a limited fraction of PS II is involved in this kind of transition. Certainly, an integration of these different concepts into a general model for regulation of photochemistry is required.

Nevertheless, this analysis provides a very good empirical fit to the experimental data reported here, and we develop a theoretically consistent hypothesis for the mechanism leading to the correlated decline of the apparent quantum efficiency of PS II with changes in the energization of the chloroplast membrane system. As will be discussed in a forthcoming paper, both photosynthetic control at the cytochrome *b-f* complex (cf. Ref. 27), and q_E quenching at PS II, may play a part in the mechanism that regulates the rate of the light-dependent reactions of photosynthesis to match that of carbon metabolism.

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Appendix

According to our hypothesis, the total yield of photochemistry and fluorescence during photosynthesis should be determined by the relative proportion of PS II in the four proposed states. At any steady state there may be: *a*, PS II_0; *b*, PS II_0^-; *c*, PS II_e, and *d* PS II_e^-, where $a+b+c+d=1$. The quanta absorbed by each of the four states should be directly proportional to its concentration. However, because of net energy transfer between centers, the proportion of quanta de-excited by a particular center may be somewhat more or less than it receives. Significant net transfer should occur from PS II_0^- to other centers which are better traps. We used coefficients α and α' to describe these transfers of energy. Thus, the total fluorescence emitted by all of the PS II units is,

$$F = \alpha' a \Phi'_{F_a} + \alpha b \Phi'_{F_b} + \alpha' c \Phi'_{F_c} + \alpha' d \Phi'_{F_d} \quad \text{(A1)}$$

and the average photochemical efficiency is,

$$\Phi_s = \alpha' a \Phi'_{P_a} + \alpha' c \Phi'_{P_c} \quad \text{(A2)}$$

The coefficients α and α' are complementary, and to satisfy conservation, these must be such that $\alpha' a + \alpha b + \alpha' c = \alpha' d = 1$. Thus, $\alpha' = (1-\alpha b)/(1-b)$.

We can express α as a function of b, by applying a theoretical model first proposed by Joliot and Joliot [28]; $\alpha = (1-p)/(1-pb)$, where p is a function of the efficiency of trapping at an open center and the yield of transfer out of a closed center (see Ref. 29). The appropriate value of p for the leaves used in our study is not known, but it should be possible to measure p by analysis of fluorescence induction curves of DCMU-poisoned leaves [29]. For example, $p = 0.4$ is typical of spinach chloroplasts with high cation concentration [29].

The fluorescence and photochemical yields for isolated PS II centers in each of the four proposed states were calculated using equations derived by Butler [17]:

$$\Phi_F = \frac{k_F}{k_F + k_D + k_T} \frac{1}{1 - \phi_T \phi_t} \quad \text{(A3)}$$

and

$$\Phi_P = \phi_T \frac{\phi_P}{1-\phi_t} \qquad \text{(A4)}$$

where ϕ_T, ϕ_t and ϕ_P are the probabilities for an exciton in the antenna being trapped by a reaction center and for a trapped exciton either returning to the antenna or resulting in photochemistry, respectively. As defined in Ref. 17, $\phi_T = k_T/(k_T + k_D + k_F)$, $\phi_t = k_t/(k_t + k_d + k_p)$, and $\phi_P = k_p/(k_p + k_d + k_t)$. Refer to Fig. 4 (inset) for identification of the constants, and a schematic diagram of the processing of absorbed quanta by PS II. Constants for decay processes were set as follows: $k_F = 2 \cdot 10^7\ s^{-1}$; $k_D = 10 \cdot 10^7\ s^{-1}$; $k_T = 100 \cdot 10^7\ s^{-1}$; $k_t = 50 \cdot 10^7\ s^{-1}$. The rate constant for photochemistry was set at $k_p = 100 \cdot 10^7\ s^{-1}$ for open centers and at $k_p = 0$ for closed centers, and the rate constant for non-photochemical quenching at the reaction center was set at $k_d = 0$ for PS II_0 and $k_d = 160 \cdot 10^7\ s^{-1}$ for PS II_e centers. For the calculations presented in Fig. 4, k_d was continuously varied from $k_d = (0 \Rightarrow 2.5)\ k_p$. These are similar data in Ref. 17, except (based on data in Ref. 25), a larger value of k_t was chosen. The calculations are presented in Table I.

Using these yields and Eqns. A1 and A2, we could simulate the responses of a hypothetical leaf using 'ideal' coefficients ($q'_Q = a/(a+b) = c/(c+d)$ and $q'_E = c + d$), or we could 'correct' experimental data, to examine the sensitivity of our analysis to the assumptions used. Possible complications in the interpretation of fluorescence quenching caused by variation in the F_0 level, and complications introduced by exchange of excitons between PS II units were of particular concern.

Simulations showed a 15% decrease in F_0 associated with $q_E = 0.85$ (the highest level observed in these studies), but the corresponding error ($(q_E - q'_E) \cdot 100/q'_E$) was less than 2%, and this decreased as $q_E \to 0$. Thus, the procedure used here of subtracting the F_0 level obtained in far-red light at the steady-state should not introduce large errors.

The simulated influence of energy exchange was much larger. The apparent values of q_Q and q_E were both increased relative to q'_Q and q'_E by exciton transfer, but the apparent photochemical yield also increased. Intuitively, one might propose that the apparent coefficients would reflect the distribution of quanta among the centers at steady-state, while the 'ideal' coefficients reflect the proportion of centers. This was analyzed by simulating the analysis of Fig. 3 by over the range ($1 > q'_Q > 0.6$ and $1 > q'_E > 0$) of the experimental data. The resultant plot (Fig. 6) was linear when $p = 0$, but was slightly non-linear (concave downwards), with a maximum deviation of approx. 12% at $q_E = 0.5$ (independent of the value of q_Q) when $p = 0.4$. Apparently, q_Q accurately reflects the redistribution of quanta, while there is a small non-linearity in the response of q_E if $p \neq 0$.

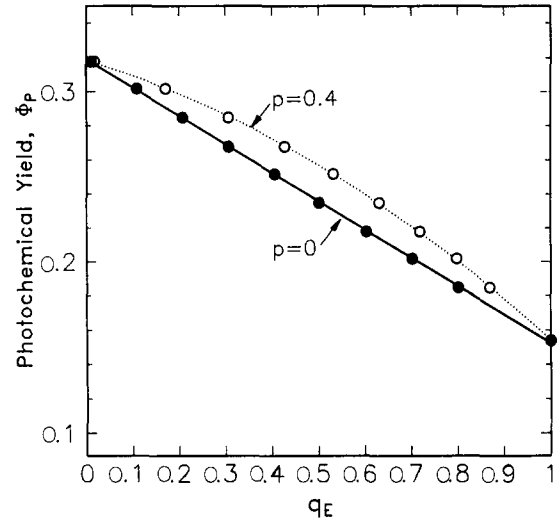

Fig. 6. The dependence of the quantum yield with 'open' PS II centers (Φ_P) on q_E based on simulations of the fluorescence and photochemical yields (using Eqns. A1 and A2) with ($p = 0.4$) or without ($p = 0$) significant transfer of energy between PS II units. The plot is based on the (uncorrected) apparent coefficients, while the photochemical and fluorescence yields were calculated by systematically varying the values of q'_E and q'_Q over the range $1 > q'_E > 0$ and $1 > q'_Q > 0.6$. The values for Φ_{P_0} and Φ_{P_e} were taken as 0.32 and 0.15, respectively, and the fluorescence yields were as given in Table I.

We also explored the effect of 'correcting' experimental data for the effect of energy exchange (assuming $p = 0.4$), since at any level, X, of variable fluorescence the coefficients α and α' may be calculated if p is known. By substituting $b = X/\alpha$ in the equation above, we obtain, $\alpha = 1 - p(1 - X)$ and $\alpha' = \alpha(1 - X)/(\alpha - X)$. Transformation of the fluorescence levels to obtain estimates of q'_E and q'_E did not substantially change the parameters of the linear regression analysis (data not shown).

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